that a legible copy must be submitted with the IDS. This Applicant has done. The Examiner's consideration of the downloaded items is therefore earnestly solicited.

In this connection, Applicant would not object if the consideration of the "other information" were recorded in an Official Action rather than a Form PTO-1449. Similarly, Applicant would not object if the Examiner chose not to list the "other information" on the face of any patent that might issue based on the present application, so long as the record reflects that this information was considered by the Examiner.

Double Patenting

The pending claims of present application have been provisionally rejected under the judicially-created doctrine of obviousness-type double patenting, as allegedly unpatentable over claims 1 through 7 and 16 through 20 of co-pending Application Nos. 10/132,642 and 10/045,790. Although the application does not agree with this rejection, in order to expedite and streamline prosecution of the present application, the applicant will submit a terminal disclaimer to overcome this rejection should a patent be issued on co-pending application 10/045,790, before the issuance of a patent on the present application.

The Rejections under 35 U.S.C. § 112

Claims 1, 4, and 6-10 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement for "other compounds that inhibit at least one of cell differentiation and cell proliferation." Applicant respectfully requests that this rejection be withdrawn upon reconsideration.

It is well established that "[a] patent need not teach, and preferably omits, what is well known in the art." *See* M.P.E.P. § 2164.05, *citations omitted.* Similarly, "[t]he fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." *Id., citations omitted.* Finally, "[t]he test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." *Id., citations omitted.*

Applicant respectfully submits that those of skill in the art understand fully what compounds are encompassed by the phrase "inhibits cell differentiation or cell proliferation," and are also able to practice the invention without undue experimentation.

Materials and methods that enable those of skill in the art to determine whether or not a given compound is within the metes and bounds of the claim are commercially available. Applicant once more respectfully draws attention to the methods that were described in the previous Response, and the supporting documentation that was submitted therewith. To reiterate briefly, the University of Massachusetts offers a license for its method of screening for cancer drugs and other drugs that inhibit or promote cell growth, cell death or cell differentiation for diseases involving Erb action. In addition, DiscoveRx Corporation of Fremont, CA markets a Hithunter™ tyrosine kinase assay to detect inhibitors of tyrosine kinase and tyrosine phosphatase, which control or regulate cellular growth, proliferation and differentiation using ß-galactosidase EFC activity. As a result, the skilled person is capable using one of these commercially available products, to determine with reasonable experimentation whether a particular compound inhibits cell differentiation or cell proliferation. Also, the existence of these test methods demonstrates that skilled persons are aware of what is meant by inhibiting cell differentiation or cell proliferation and thus this terminology is not indefinite.

The commercial availability of assay kits, and the enclosed Declaration of Dr. Rosenbloom, also demonstrates that the experimentation, if any, that must be performed to test a given compound for the required activity is of a routine nature. *See* M.P.E.P. § 2164.05, *quoted above*. Therefore, both the definition of the phrase "inhibits cell differentiation or cell proliferation" and the appropriate test methods to determine whether a compound meets this limitation are apparent to those aware of the prior art.

Finally, it is noted that the applicant has now submitted substantial evidence in support of its position and that the Examiner has submitted no evidence to rebut the applicant's evidence. It is the Examiner's burden to demonstrate lack of enablement and indefiniteness. Accordingly, without rebuttal evidence, the Examiner should withdraw the rejections.

Accordingly, it is respectfully submitted that the claims are in full compliance with the requirements of 35 U.S.C. § 112. Applicant therefore respectfully requests that the rejections under this statute be withdrawn upon reconsideration.

Claims 1 to 11 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement for the term "prevention," and also as allegedly failing to convey that the Applicant had possession of the claimed invention when the application was filed. This rejection, at least

insofar as it applies to claims 1 and 11, as amended, is respectfully traversed and reconsideration is requested for the reasons, which follow.

Independent claims 1 and 11, as amended herein, are drawn to the "reduction, treatment, or prevention of at least one adverse effect of radiation dermatitis." Applicant respectfully submits that those of skill in the art are well aware of the adverse effects of radiation dermatitis, of which several are listed in the specification on page 1 at lines 9 to 15. Moreover, since the claims no longer require the "prevention of radiation dermatitis" *per se*, but instead refer to the prevention of at least one adverse effect of radiation dermatitis, it is considered that the Examiner's arguments have been rendered moot since they applied to the total prevention of radiation dermatitis, rather than the prevention of an adverse effect of radiation dermatitis. Accordingly, for these reasons, withdrawal of the rejection is requested.

Claims 1-11 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to convey to a skilled person that the applicant had possession of the invention at the time the application was filed. Specifically, the Examiner objected to the claim limitation specifying the specific types of radiation: alpha radiation, beta radiation, gamma ray radiation, and x-ray radiation.

Claim 1 has been amended to replace "x-ray" with "fluoroscopic" to conform the language of claim 1 to the language of page 14, lines 6-9 of the specification. This amendment, however, is not considered to change the scope of the claim since "fluoroscopic radiation" is a synonym for "x-ray radiation." This rejection, at least insofar as it applies to claims 1-11, as amended, is respectfully traversed and reconsideration is requested for the reasons, which follow.

First, claim 11 does not contain the language objected to by the Examiner. Accordingly, this rejection should be withdrawn for claim 11 for this reason.

Second, applicant respectfully draws attention to page 14 of the specification at lines 6 to 9 which states that the compositions of the present invention may be employed to treat radiation dermatitis resulting from exposure to one or more of alpha radiation, beta radiation, gamma ray radiation and fluoroscopic radiation. Accordingly, there is a clear written description of these limitations of claim 1 in the specification as originally filed. Thus, Applicant respectfully submits that these limitations are supported by the original application and that the rejection under 35 U.S.C. §112, first paragraph, should be withdrawn.

Claims 1-11 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. More specifically, the Examiner has objected to the use of the phrase, "compounds that inhibit at least one of cell differentiation and cell proliferation" in these claims. This rejection is respectfully traversed and reconsideration is requested for the reasons, which follow.

First, claim 11 does not include the limitation objected to by the Examiner and thus, for this reason alone, the rejection of claim 11 under 35 U.S.C. §112, second paragraph, should be withdrawn.

Regarding the rejection of the phrase "compounds that inhibit at least one of cell differentiation and cell proliferation" for alleged indefiniteness, it is likewise well established that:

> The essential inquiry pertaining to this requirement is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:
>
> (A) The content of the particular application disclosure;
>
> (B) **The teachings of the prior art**; and
>
> (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

M.P.E.P. § 2173.02, *emphasis supplied.* Moreover, as stated in M.P.E.P. § 2173.04, "[b]readth of a claim is not to be equated with indefiniteness."

Materials and methods that enable those of skill in the art to determine whether or not a given compound is within the metes and bounds of the claim are commercially available. Applicant once more respectfully draws attention to the methods that were described in the previous Response, and the supporting documentation that was submitted therewith. To reiterate briefly, the University of Massachusetts offers a license for its method of screening for cancer drugs and other drugs that inhibit or promote cell growth, cell death or cell differentiation for diseases involving Erb action. In addition, DiscoveRx Corporation of Fremont, CA markets a Hithunter™ tyrosine kinase assay to detect inhibitors of tyrosine kinase and tyrosine phosphatase, which control or regulate cellular growth, proliferation and differentiation using ß-

galactosidase EFC activity. As a result, the skilled person is capable using one of these commercially available products, to determine with reasonable experimentation whether a particular compound inhibits cell differentiation or cell proliferation. Also, the existence of these test methods demonstrates that skilled persons are aware of what is meant by inhibiting cell differentiation or cell proliferation and thus this terminology is not indefinite.

In addition, the applicant did a brief internet search and located a list of 56 assays for determining cell proliferation on the Medline database. A copy of the list of 56 assays is enclosed for the convenience of the Examiner. Also enclosed is an excerpt from the Apoptosis Special Interest Site which, under the section "Test Principle" indicates that cell proliferation assays are of importance for routine applications, thereby indicating that this is considered to be routine testing. This additional evidence also clearly supports the applicant's position.

Applicant respectfully submits that the commercial availability of these materials and methods in the prior art is ample evidence that the scope of the claims is clear to those of skill in the art. In order to confirm this, applicant encloses a Declaration of Dr. Richard Rosenbloom, which states that a skilled person can use of these commercially available materials and methods to routinely determine whether a particular compound is a compound that inhibits cell differentiation or cell proliferation.

Accordingly, withdrawal of the rejection of claims 1-11 under 35 U.S.C. §112 for these reasons is requested.

The Rejections under 35 U.S.C. § 103

Claims 1 to 8 and 10 have been rejected as allegedly obvious over U.S. Patent No. 6,162,801, issued to Kita (hereinafter "Kita"), the article by Darr, D., *et al.*, in the *British Journal of Dermatology*, 1992, 127, 247-253 (hereinafter "Darr"), and U.S. Patent No. 5,972,359, issued to Sine et al. (hereinafter "Sine"). Claims, 1, 9, and 11 have also been rejected as allegedly obvious over Kita, Darr, and Sine in view of U.S. Patent No. 6,048,886, issued to Neigut (hereinafter "Neigut"), U.S. Patent No. 5,876,737, issued to Schönrock et al. (hereinafter "Schönrock"), and U.S. Patent No. 5,952,391, issued to Gers-Barlag et al. (hereinafter "Gers-Barlag"). Applicants respectfully traverse these rejections for the reasons set forth below.

1. The rejection citing Kita, Darr, and Sine

It is well-established that

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.
>
> The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure.

M.P.E.P. § 2143, *citation omitted.* Applicant respectfully submits that the teachings of Kita, Darr, and Sine do not teach or suggest all the claim limitations. Nor do these references provide a reasonable expectation for the success of Applicant's invention.

Briefly, as noted in the Official Action, Kita, Darr, and Sine set forth compositions and methods for treating damage caused by exposure to ultraviolet radiation. Damage caused by ultraviolet radiation is different both in level **and in type** from damage caused by ionizing radiation. The distinction between radiation dermatitis, a permanent condition caused by exposure to ionizing radiation, and solar erythema, or sunburn, a temporary condition caused by overexposure to UV radiation, is well documented in the art.

Furthermore, alpha radiation, beta radiation, gamma ray radiation and fluoroscopic radiation, the types of radiation specifically recited in claim 1, are all types of ionizing radiation. The ionizing radiation types specifically recited in claim 1 do not appear in any of the cited references. Applicant respectfully submits that there can be no reasonable expectation that treatments previously known to be effective for UV damage, such as sunburn, would, in combination, be successful as a treatment for radiation dermatitis caused by ionizing radiation since ionizing radiation is well-known to cause far more severe injuries than ultraviolet radiation. Therefore, Applicant respectfully requests that the rejection over Kita, Darr, and Sine be withdrawn upon reconsideration.

Although Applicant believes that the above reasoning is sufficient to overcome the rejection under § 103 citing Kita, Darr, and Sine, the following supplemental reasons and evidence are presented.

Radiation dermatitis is considered to be a serious, irreversible injury, which results primarily from exposure of the skin to high-energy **ionizing radiation** such as alpha, beta, gamma and x-ray or fluoroscopic radiation. See the publication "What is Ionizing Radiation" (6 pages) taken from http://tis.eh.doe.gov/ohre/roadmap/achre/into_9_1.html, enclosed with the previous Response. Ionizing radiation is defined therein as any form of radiation that has enough energy to knock electrons out of atoms or molecules, creating ions. See page 2 of "What is Ionizing Radiation."

Ionizing radiation is thus distinguishable from, for example, actinic radiation, i.e. ultraviolet or violet radiation since ionizing radiation has significantly more energy than ultraviolet radiation. Ultraviolet radiation also does not have sufficient energy to knock electrons out of atoms or molecules creating ions. As a result, the types of damage to the skin caused by ionizing radiation, is of a different kind and severity than is typically caused by exposure of the skin to relatively lower energy actinic radiation such as ultraviolet radiation.

The article "Sunburn" by James Foster, MD, MS, of the Alverado Hospital Medical Center (12 pages) found at http://www.emedicine.com/EMERG/topic798.htm points out on page 1 of 12 that the most common deleterious effect of exposure to ultraviolet radiation is sunburn or solar erythema. This article also points out that long-term sun exposure may lead to the development of cancers such as basal cell carcinoma, squamous cell carcinoma and malignant melanoma. See page 2 of 12 of "Sunburn." These are the types of injury caused by exposure to the relatively lower energy ultraviolet or actinic radiation.

The article, "Radiation Safety Answers, Answer to Question #13," by Aggie Barlow, Radiation Safety Officer, Yale University (4 pages) found at http://www.yale.edu/oehs/rdsfq13.htm discusses the harmful effects of one type of higher energy ionizing radiation, namely, x-ray radiation. X-ray radiation has approximately 1000 times the energy of ultraviolet radiation since the wavelength of x-ray radiation is approximately 1000 times shorter than the wavelength of ultraviolet radiation and the energy of radiation is inversely proportional to the wavelength of the radiation. The article "Radiation Safety Answers" discusses three types of biological effects of x-ray beams on pages 2-3 of the article: (1) reversible changes, (2) conditional reversible changes, and (3) irreversible changes. Among the reversible changes caused by x-ray radiation is erythema, similar to the most common injury caused by exposure to ultraviolet radiation, namely, solar erythema, as discussed above. See

page 2 of "Radiation Safety Answers." Among the irreversible changes caused by x-ray radiation is radiation cancer, again similar to the long-term effect of ultraviolet radiation of causing certain types of cancer as mentioned above.

However, a third, totally different type of injury caused by x-ray radiation, also considered irreversible, is radiation dermatitis, and, a fourth, totally different type of injury caused by x-ray radiation is chronic radiation dermatitis. See page 3 of "Radiation Safety Answers." Thus, "Radiation Safety Answers" makes it clear that (1) radiation dermatitis is a different injury than erythema and radiation cancer, that (2) that ionizing x-ray radiation can cause radiation dermatitis, and (3) that radiation dermatitis is not an injury typically caused by ultraviolet radiation. For example, exposure to ultraviolet radiation generally does not cause the irreversible damage associated with radiation dermatitis, such as permanent destruction of hair or sweat glands or of skin cells.

Accordingly, from the above publications it can be concluded that radiation dermatitis is a known illness, which is considered irreversible, and which is different than the damage that is caused by exposure to ultraviolet radiation. It can also be concluded that the mechanism causing radiation dermatitis is most likely associated with the knocking out of electrons from, for example, skin cells or elements thereof due to exposure to high-energy ionizing radiation, which phenomena does not occur as a result of exposure to the significantly lower energy ultraviolet radiation. This demonstrates that, contrary to the Examiner's assertion, radiation dermatitis is a different kind of injury than injury caused by ultraviolet radiation and not just a different degree of injury as the Examiner suggests.

Therefore, it is considered that the present claims, which are specifically drawn to the treatment of radiation dermatitis caused by ionizing radiation, clearly distinguish the present invention from all of the prior art relied upon by the Examiner. The main distinction is that ionizing radiation causes a different, more harmful type of damage, characterized as "radiation dermatitis," than does ultraviolet radiation due to its higher energy state. Thus, a skilled person would not apply the teachings of prior art relating to the treatment of sunburn to the treatment of radiation dermatitis which results from exposure to alpha, beta, gamma or x-ray radiation, as claimed, since it is apparent from, for example, "Radiation Safety Answers" that radiation dermatitis caused by these types of high-energy radiation is a different type of injury than the types of injury that is typically caused by exposure to the relatively lower energy ultraviolet

radiation. Moreover, it is clear from the prior art that the skilled person would have no expectation of success for the claimed invention since the article, "Radiation Safety Answers" classifies radiation dermatitis as an irreversible injury thereby indicating that it is the belief of skilled persons that radiation dermatitis cannot be treated in the same manner as common sunburn or other injuries resulting from exposure to ultraviolet radiation. Accordingly, all claims of the present application are believed to be patentable over the cited prior art for at least this reason.

Kita discloses an ophthalmic or dermatological composition comprising vitamin D for treating disturbed metabolism in eye tissues and protecting skin against UV radiation. However, Kita does not teach the use of the composition for treating radiation dermatitis or radiation injury caused by ionizing radiation such as the types claimed in claims 1-10 of the present application. In fact, Kita disavows treatment of radiation damage to the skin when it states that "[I]n U.S. Pat. No 4,610,978, Dikstein et al disclose active vitamin D as a skin cream for treating the skin. By contrast, the present invention is a cosmetic or other dermatological composition which uses vitamin D to protect the skin against UV radiation." Thus, a skilled person familiar with the various types of radiation would not expect the composition of Kita to be useful to treat radiation dermatitis and also has no reason to conclude that this composition would protect the skin against ionizing radiation, which has a different mechanism for causing damage to the skin than UV radiation.

Darr sets forth methods of increasing cutaneous levels of vitamin C, and of protecting porcine skin from UV damage, by applying vitamin C or its isomers in a solution of water and propylene glycol thickened with hydroxypropylcellulose. *See* page 247, column 2 of Darr.

Sine discloses a composition for regulating a skin condition comprising a particulate material such as TiO_2 (col. 4, lines 41-42 of Sine) and a carrier containing vitamin E acetate, vitamin A, D-panthenol, acrylic copolymer and PEG stearate (col. 5, line 53 of Sine). According to Sine, regulating a skin condition includes regulating visible and/or tactile discontinuities in skin, including but not limited to visible and/or tactile discontinuities in skin texture and/or color, more especially discontinuities associated with skin aging. Such discontinuities may be induced or caused by internal and/or external factors. Extrinsic factors include ultraviolet radiation (e.g., from sun exposure), environmental pollution, wind, heat, low humidity, harsh surfactants, abrasives, and the like. Intrinsic factors include chronological aging and other biochemical

changes from within the skin." (Col. 3, lines 25-36). Nowhere does Sine teach using the composition to treat radiation dermatitis. Rather, Sine relates again to a purely cosmetic composition for the skin. Sine is mainly concerned with "masking" the skin discontinuity caused by aging, UV radiation, wind, pollution, heat, low humidity, and harsh surfactant among many other factors.

The composition of Sine is used to impart an essentially immediate visual improvement in skin appearance (col. 2, lines 29-32 of Sine). The compositions of Sine are characterized by their contrast ratio and % transmittance or coverage index (col. 2, lines 32-34 of Sine). In fact, Sine goes extra length to discuss the importance of refractive index of the particulate material. Clearly, UV radiation is just one of the more than 10 factors that can cause such skin discontinuities. Furthermore, Sine even admits that "it is believed that this acute skin appearance improvement results at least in part from therapeutic coverage or masking of skin imperfections by the particulate material." (Col. 3, lines 19-23 of Sine)

Furthermore, the combination of the teachings of Kita and Sine would result a composition unsuitable for the objective of Kita. Kita uses its composition for ophthalmic treatment. Clearly, the TiO_2 particulates contained in the composition of Sine would result in a composition unsuitable to be used in ophthalmic treatment since such particulates would cause abrasive damage to the eyeball. Therefore, a skilled person reading these two references would not be motivated to combine the teachings of these two references.

The applicant has now submitted significant evidence in support of its position that radiation dermatitis is a different type of injury than those caused by ultraviolet radiation. The Examiner has provided no evidence rebutting the applicant's position. Accordingly, for this additional reason, the rejection under 35 U.S.C. §103(a) should be withdrawn.

Finally, all of the dependent claims currently pending in the present application ultimately depend from independent claim 1. Applicant respectfully submits that, because independent claim 1 is not obvious over the cited references, dependent claims 2 to 8 and 10 are also not obvious. Accordingly, favorable reconsideration and withdrawal of the rejection of claims 1-8 and 10 under 35 U.S.C. § 103(a) over Kita, Darr and Sine et al. is respectfully requested.

2. The rejection of claims 1, 9 and 11 Under 35 U.S.C. §103(a) citing Kita, Darr, Sine, Neigut, Schönrock, and Gers-Berlag.

Referring once more to M.P.E.P. § 2143, quoted in full above, Applicant respectfully submits that the Official Action has not set forth a *prima facie* case of obviousness for claims 1, 9, and 11 over the cited references.

Briefly, the prior art does not teach or suggest every claim limitation. Again, claim 1 specifically recites that the radiation dermatitis is caused by alpha radiation, beta radiation, gamma ray radiation or fluoroscopic (x-ray) radiation, whereas the cited references all pertain to the treatment of UV-induced damage.

Second, the cited references provide no reasonable expectation for the success of Applicant's claimed invention. As documented above, damage caused by ultraviolet radiation is different in both level **and kind** from damage caused by ionizing radiation. Therefore, a skilled person reading the references cited by the Examiner has no reasonable expectation that ingredients known as treatments for UV-induced damage would be effective to prevent or alleviate the symptoms of radiation dermatitis caused by ionizing radiation.

The same arguments given above also apply to Neigut et al., Schonrock et al., and Gers-Barlag et al., since all of these references relate to the treatment of UV radiation induced damage, these references do not mention radiation dermatitis and these references contain no teaching or suggestion that the disclosed compositions would be effective to treat damage caused by ionizing radiation.

Therefore, Applicant respectfully submits that the Official Action has not set forth a *prima facie* case of obviousness. Accordingly, Applicant respectfully requests that the rejection under § 103 citing Kita, Darr, Sine, Neigut, Schönrock, and Gers-Berlag,be withdrawn upon reconsideration.

Although Applicant believes that the above reasoning is sufficient to overcome the present rejection, the following supplemental reasons and evidence are presented.

Neigut discloses a composition comprising vitamins A, D, E, ascorbyl palmitate, α-lipoic acid, and an antioxidant enzyme, superoxide dismutase, in a corn oil vehicle, and a method of treating skin conditions such as psoriasis (see col. 1, lines 18-19, and col. 7, lines 40-60 of Neigut). Applicant respectfully draws attention to the fact that Neigut uses vitamin D without

specifying which vitamin D it refers to. There are several forms of vitamin D and thus it is not clear that the vitamin D in Neigut is, in fact, vitamin D_3.

Schönrock uses a composition including vitamin E acetate and hydroxypropylmethyl-cellulose for cosmetic and topical dermatological treatment (col. 14, lines 1-45, col. 1, lines 6-9 of Schönrock).

Gers-Barlag et al. discloses the use of quercetin against UV-induced decomposition of dibenzoylmethane and its derivatives (col. 4, lines 45-61, and col. 14, lines 25-40 of Gers-Barlag).

First, the combination of all the cited references lacks an element that is specifically recited in claim 1, that is, treating radiation dermatitis caused by one or more radiations selected from the group consisting of alpha, beta, gamma-ray and fluoroscopic radiations.

Furthermore, the skilled person would not combine some of the references as the Examiner suggests. For example, one reading Gers-Berlag would have no motivation to add quercetin to the composition of, e.g. Neigut, or Kita, since the compositions of Neigut and Kita do not contain dibenzoylmethane, for which quercetin functions as a protecting agent according to Gers-Berlag (col. 7, line 62 to col. 8, lines 67 of Gers-Barlag). Thus, if the composition does not contain dibenzoylmethane, there is no reason to add quercetin as taught by Gers-Berlag.

The skilled person would also not combine Sine with the other references, as discussed above, since Sine is primarily concerned with "hiding" skin imperfections by choosing ingredients with proper reflectivity index (col. 1, line 10-13, col. 2, lines 23-26 of Sine). As discussed above, for example, a proposed combination of Kita and Sine would be a problem since TiO_2 is not compatible with an ophthalmic composition.

Finally, the Examiner has picked and chosen specific ingredients from different compositions disclosed for use for different purposes from not less than six references to arrive at a rejection of the composition of claim 11. It is only with the use of hindsight that these six specific ingredients from these six references can be chosen. For example, to arrive at the invention as claimed in claim 11 from Neigut, considered to be the closest of the six references to the composition of claim 11), a skilled person has to:

1) choose vitamin D_3 out of all the possible vitamin D's;

2) add quercetin to the composition even though it does not contain dibenzoylmethane type material;

3) use a dispersion of vitamin A and D_3;

4) use vitamin E acetate instead of vitamin E; and

4) determine the specific amounts for each of vitamin A, vitamin D_3, vitamin E acetate, ascorbyl palmitate, quercetin, α-lipoic acid to be used in the particular composition.

Clearly, the skilled person would not arrive at the specific composition of claim 11 of the present application due to the teachings mentioned above and the necessity of making so many modifications to the cited prior art composition. Also, there is no motivation in the various references to make the required modifications. Therefore, claim 11 is considered to be patentable over the cited references for this additional reason.

For the foregoing reasons, claims 1, 9 and 11 are believed to be patentable in view of Kita, Sine, Darr, Neigut, Schönrock, and Gers-Barlag. Accordingly, withdrawal of the rejection under 35 U.S.C. §103 is respectfully requested.

Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully submits that all of the pending claims are in condition for allowance and respectfully requests a favorable Office Action so indicating.

Respectfully submitted,

Kevin J. Dunleavy
Registration No. 32,024

Dated: March 13, 2003

Appendix: Claim 1 as Amended, with Revisions Marked

Enclosure: Declaration of Dr. Richard Rosenbloom

KNOBLE & YOSHIDA, LLC (Customer No. 21,302)
Eight Penn Center, Suite 1350
1628 John F. Kennedy Blvd.
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APPENDIX: CLAIMS AS AMENDED, WITH REVISIONS MARKED

1. (Twice amended) A method for the ~~prevention, reduction or~~ , treatment, or prevention of at least one adverse effect of radiation dermatitis caused by one or more types of radiation selected from the group consisting of alpha radiation, beta radiation, gamma ray radiation and ~~x-ray~~fluoroscopic radiation, comprising the step of applying to an area of skin which has been or will be exposed to said one or more types of radiation, a topical composition which comprises:

an amount of one or more compounds that inhibit at least one of cell differentiation and cell proliferation, metabolites thereof, and pharmaceutically acceptable salts thereof, which is effective, when administered topically in the topical composition to inhibit at least one of cell differentiation and cell proliferation, and

an effective amount of one or more antioxidants~~, and~~ or pharmaceutically acceptable salts thereof,

formulated in a pharmaceutically acceptable carrier for a topical composition.

#9
m.m.
3/27/03

PATENT
Attorney Docket No.: QUIG-1006US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Dr. Richard Rosenbloom

U.S. Application No.: 09/993,003 — Group Art Unit: 1617

Filed: November 6, 2001 — Examiner: Hui, San Ming R.

For: COMPOSITION AND METHOD FOR PREVENTION, REDUCTION, AND TREATMENT OF RADIATION DERMATITIS

DECLARATION OF DR. RICHARD ROSENBLOOM PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner of Patents & Trademarks
Washington, D.C. 20231

Sir:

1. I, Richard Rosenbloom, M.D., Ph.D., hereby declare as follows:

2. I am the inventor of the above-identified application. My *curriculum vitae* is attached hereto. I am a medical doctor with an additional doctorate degree and have significant experience in fields relating to endocrinology and neurology. I have worked experience relating to the treatment of the human body for cancer, pain, diabetes, and complications of diabetes.

3. I have reviewed the relevant portions of the specification, drawings and claims of U.S. Application No. 09/993,003.

4. It is my opinion that a person of ordinary skill in the art would be capable of making and using the invention claimed in any one of claims 1-11 of the above-identified application using a combination of common general knowledge known to such a person of ordinary skill in the art and the disclosure contained in the specification, drawings and claims of the application as originally filed.

5. More specifically, a person of ordinary skill in the art is familiar with the fact that the University of Massachusetts offers a license for a method of screening for cancer drugs and other drugs that inhibit or promote cell growth, cell death or cell differentiation for diseases involving Erb action. The person of ordinary skill in the art is also aware that DiscoveRx Corporation of Fremont, CA markets a Hithunter™ tyrosine kinase assay to detect inhibitors of tyrosine kinase and tyrosine phosphatase, which control or regulate cellular growth, proliferation and differentiation.

6. The person of ordinary skill in the art is capable using one of these commercially available products, to determine with reasonable experimentation whether a particular compound inhibits cell differentiation or cell proliferation. Also, the existence of these test methods demonstrates that skilled persons are aware of what is meant by inhibiting cell differentiation or cell proliferation.

7. Inhibition of cell differentiation or cell proliferation can be determined by a simple comparative test wherein a control sample is employed to determine the rate of cell differentiation or cell proliferation. A second sample, containing the compound of interest is tested under the same conditions as the control sample and the rate of cell differentiation or cell proliferation is determined. If the rate of cell differentiation or cell proliferation has been reduced, relative to the control sample, then the tested compound meets the requirement of the claims. This type of testing is routine experimentation and can be accomplished for a specific compound in a short time period using commercially available test kits and methods.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that the statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001

U.S. Application No.: 08/473,974

of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

By: _____
Richard Rosenbloom, M.D., Ph.D.

Dated: 3/13/03

RICHARD A. ROSENBLOOM, M.D., Ph.D.
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EXPERIENCE:

May 2000 – Present

Quigley Pharma, Inc.
Executive Vice President/ Chief Operating Officer

March 1997 – May 2000

Independent Consultant Clinical Drug Development
Safety, protocol development, medical marketing, FDA liaison. ENDO Pharmaceuticals, Sanwa Pharmaceuticals

March 1997 – July 1999

Asta Medica, Inc., Hackensack, N.J.
Director, Medical Affairs

Therapeutic areas of experience:
Diabetes and diabetes complications, neuropathy, pain and cancer.

Responsibilities :
Safety, pharmacovigilence, protocol development, clinical Development plans, opinion leader and investigator recruitment, budget negotiation, in-service training and medical marketing. Managed up to 15 employees.

Selected for International Management Training.
Coordinated one International study.

May 1992 – March 1997

Biotrax Clinical Research, Inc./Comprehensive Clinical Research, Inc., Chadds Ford, PA
Medical Director

Responsible for the design, placement and execution of clinical research studies in the fields of: endocrinology (completed the only successful aldose reductase inhibitor study for diabetic neuropathy), neurology and rheumatoid arthritis. Managed up to 4 employees.

February 1983 – May 1992	**Human Genetic Disease Habilitative Services,** Linwood, N.J. **Director of Research** Designed and conducted research on Prader-Willi Syndrome, neurotransmitter precursors and the environmental influences on the behavior and rehabilitation of paraplegics.
1980 – 1981, 1983	**Institute of Nutritional Medicine**, Philadelphia, PA **Director and Research Coordinator** (Part of this time was spent in graduate school) Responsibilities included administration, implementation of pilot studies, in-service training and clinical nutrition services.

EDUCATION:

Undergraduate Education:	1969 – 1973
Medical School:	1974 – 1978
Pan American Fellow:	1979
Graduate Education:	
Ph. D.	1983

PROFESSIONAL MEMBERSHIPS:

American Diabetes Association Professional Section, Member of Council on Complications, American Diabetes Association, Member American Association of Clinical Endocrinologists, Member American Academy of Pharmaceutical Physicians, Member American Pain Society, American Association of Pharmaceutical Scientists, Southern Medical Association.

CONTINUING EDUCATION:

Carcinogenicity and Toxicology of Pharmaceuticals
Complications of Diabetes Mellitus and Diabetic Foot Management
Designing Better Drugs and Clinical Trials for SEPSIS/SIRS
Drug Safety Assessment in Clinical Trials
Neuropathic Pain Management
GCP's
International Management Training

PHARMACEUTICAL RESEARCH EXPERIENCE OF:

RICHARD A. ROSENBLOOM, M.D., Ph.D.

DIRECTED THE FOLLOWING STUDIES:

Zenerestat	Phase IIb aldose reductase inhibitor for diabetic neuropathy
Alpha lipoic acid	Phase II antioxidant for diabetic neuropathy International Study
PDGF	Phase II platelet derived growth factor for diabetic foot ulcers
	Sustained release analgesic safety study for non-malignant pain
	Cytotoxic bioequivalence study in cancer patients

WROTE CLINICAL DEVELOPMENT PLANS FOR:

LH/RH agonist implant for prostate cancer

Anti-oxidant for complication of diabetes

Immunosuppressant for rheumatoid arthritis

CONDUCTED FEASIBILITY STUDIES FOR:

Anti-Alzheimer's drug

Anti-fungal drug

Drug for the Treatment of Sepsis/SIRS

OTHER DUTIES:

Safety

Protocol Development

Organizing opinion leaders and investigators

Selecting CROs

Negotiating budgets

CRA supervision

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PROFILE

Cell Proliferation Assays

Company	Product	Method/ Measurement	Suggested Use
Alexis Biochemicals (800) 900-0065 www.alexis-corp.com	Cell Counting Kit-8	Metabolic activity of viable cells via WST-8 reduction to formazan salt; colorimetric assay.	Quantification of viable cell number in proliferation and cytotoxicity assays.
Amersham Pharmacia Biotech (800) 526-3593 www.apbiotech.com	Cell Proliferation ELISA	DNA synthesis measured by BrdU incorporation; anti-BrdU mAb/HRP-based detection.	Quantification of cell number, proliferation, and cytotoxicity.
	Cell Proliferation Immunocytochemistry	DNA synthesis measured by BrdU incorporation; anti-BrdU mAb/HRP-based detection.	Immunocytochemistry suitable for both in vivo and in vitro tissue labeling.
	Thymidine Uptake [^{14}C] Cytostar-T Assay	DNA synthesis by [^{14}C] thymidine uptake	Real-time measurement of DNA synthesis.
	[^{3}H] Thymidine Uptake Assay Kit	DNA synthesis measured by scintillation proximity assay (SPA) of [^{3}H] thymidine incorporation.	High-throughput screening of cell proliferation.
BD Biosciences (800) 448-2347 www.bd.com	FastImmune Anti-BrdU with DNase	Simultaneous measurement of cell surface markers and intracellular BrdU incorporation.	Individual peripheral blood mononuclear cells (PBMCs); quantification of cell proliferation, phenotypic identification of proliferating cells, study of activation markers, and

			intracellular cytokine production.
BioVisi n Inc. (800) 891-9699 **www.biovisionlabs.com**	Quick Cell Proliferation Assay Kit	Metabolic activity of viable cells via WST-1 reduction to soluble formazan salt; colorimetric assay.	Quantification of cell number, proliferation, viability, and cytotoxicity.
	Live/Dead Cell Staining Kit	Live cells stained with "Cyto-dye"; dead cells stained with propidium iodide.	Quantification of cell number, proliferation, viability, and cytotoxicity.
	ApoSENSOR ATP Determination Kit	Metabolic activity; bioluminescent detection of ATP.	Detects 10–100 cells/well; can be fully automated for high-throughput research.
BioWhittaker (800) 344-6618 **www.clonetics.com**	LumiTech's ViaLight HS Assay	Metabolic activity; bioluminescent detection of ATP.	For use with mall ce numbers—10 cells/w needed; quantificatio of mammalian cell proliferation.
	LumiTech's ViaLight HT Assay	Metabolic activity; bioluminescent detection of ATP.	For high-throughput needs using 100 cells/well; quantification of cell proliferation.
	LumiTech's ViaLight MDA Assay	Metabolic activity; bioluminescent detection of ATP.	For noneukaryotic cells.
Chemicon International Inc. (800) 437-7500 **www.chemicon.com**	MTT Cell Growth Assay Kit	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity.
	Cell Proliferation Assay Kit	Metabolic activity of viable cells via WST-1 reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity.
Dojindo Molecular Technologies Inc. (877) 987-2667 **www.dojindo.com**	Cell Counting Kit-8	Metabolic activity of viable cells via WST-8 reduction to formazan salt; colorimetric assay.	Quantification of viab cell number in proliferation and cytotoxicity assays.
		Live cells stained	Cell viability

	Cellstain Double-Staining Kit	with calcein-AM; dead cells label with propidium iodide.	determination by fluorescent microscop or flow cytometry.
Intergen (800) 431-4505 **www.intergenco.com**	ProCheck Cell Viability Assay	Metabolic activity of viable cells—XTT reduction to formazan salt; colorimetric	Cell viability, cytotoxicity, quantification of cell proliferation
MBL International Corp. (800) 200-5459 **www.mblintl.com**	Quick Cell Proliferation Assay Kit (manufactured by BioVision)	Metabolic activity of viable cells via WST-1 reduction to formazan salt.	Quantification of viab cell number in proliferation and cytotoxicity assays.
Molecular Probes Inc. (800) 438-2209 **www.probes.com**	CyQUANT Cell Proliferation Assay Kit	DNA content measured using CyQUANT GR dye.	Quantification of cell number in culture.
	Vybrant MTT Cell Proliferation Assay Kit	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity, necrosis, and apoptosis.
Oncogene Research Products (800) 662-2616 **www.oncresprod.com**	BrdU Cell Proliferation Assay	DNA synthesis measured by BrdU incorporation; ELISA-based chemiluminescent detection.	Cell populations; quantification of DNA synthesis during cell proliferation.
	BrdU Proliferation Assay—HTS	DNA synthesis measured by BrdU incorporation; ELISA-based colorimetric detection.	Quantification of cell number, proliferation, viability, and cytotoxicity.
	PCNA (Proliferating Cell Nuclear Antigen) ELISA	PCNA quantified using biotinylated anti-PCNA mAb.	PCNA quantification; detection of transition from nonproliferating to proliferating cell population.
	Rapid Cell Viability Assay	Metabolic activity of viable cells via WST-1 reduction to formazan salt; colorimetric assay.	Quantification of cell number, proliferation, viability, and cytotoxicity.
	BrdU IHC System	DNA synthesis measured by BrdU incorporation; detection using anti-BrdU mAb.	Immunohistochemistr paraffin sections.

P rkin Elm r Lif Scienc s (800) 551-2121/ (617) 482-9595 www.perkinelmer.com/lifesciences	CytoLux Assay Kit	Metabolic activity; bioluminescent detection of ATP.	Quantification of mammalian cell proliferation.
Phoenix Flow Systems Inc. (800) 886-FLOW www.phnxflow.com	ABSOLUTE-S SBIP Cell Proliferation Assay Kit (offered jointly with Molecular Probes)	DNA synthesis measured by BrdU incorporation using Strand Break Induced Photolysis (SBIP) methodology; break sites identified with BrdU incorporation.	Quantification of cell proliferation by analysis of DNA replication and cellula DNA content/cell cycl position by flow or image cytometry.
Promega (800) 356-9526 www.promega.com	CellTiter 96 Non-Radioactive Cell Proliferation Assay	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity, attachment, chemotaxis, and apoptosis.
	CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay	Metabolic activity of viable cells via MTS reduction to soluble formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity, attachment, chemotaxis, and apoptosis.
	CellTiter 96 AQueous One Solution Cell Proliferation Assay	Metabolic activity of viable cells via MTS reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity, attachment, and chemosensitivity.
	CellTiter-Glo Luminescent Cell Viability Assay	Metabolic activity; bioluminescent detection of ATP using luciferin and thermostable luciferase.	Quantification of viab cell number, cytotoxicity, proliferation, and attachment.
R&D Systems (800) 343-7475 www.rndsystems.com **Roche Molecular Biochemicals** (800) 428-5433 biochem.roch .com	TACS MTT Cell Proliferation and Viability Assay (manufactured by Trevigen)	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, cytotoxicity, necrosis, and apoptosis.
		Single-cell	

In Situ Cell Proliferation Kit, FLUOS	proliferation assay; direct immunofluorescence staining.	Detection of BrdU-labeled DNA in a single proliferating ce
BrdU Labeling and Detection Kit I	Single-cell proliferation assay; direct immunofluorescence staining.	Detection of BrdU-labeled DNA in a single proliferating ce
BrdU Labeling and Detection Kit II	Single-cell proliferation assay; indirect immunostaining method.	Detection of BrdU-labeled DNA in a single proliferating ce
BrdU Labeling and Detection Kit III	DNA synthesis measured by BrdU incorporation; ELISA with colorimetric detection.	Quantification of DNA synthesis during cell proliferation.
Cell Proliferation Kit I (MTT)	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell number, proliferation, viability, cytotoxicity.
Cell Proliferation Kit II (XTT)	Metabolic activity of viable cells via XTT reduction to soluble formazan salt; colorimetric assay.	Quantification of cell number, proliferation, viability, and cytotoxicity.
Cell Proliferation Reagent WST-1	Metabolic activity of viable cells via WST-1 reduction to soluble formazan salt; colorimetric assay.	Quantification of c ll number, proliferation, viability, and cytotoxicity.
Cell Proliferation ELISA, BrdU (colorimetric)	DNA synthesis measured by BrdU incorporation; ELISA with colorimetric detection.	Quantification of DN synthesis during cell proliferation.
Cell Proliferation ELISA, BrdU (chemiluminescent)	DNA synthesis measured by BrdU incorporation; ELISA with chemiluminescent detection.	Quantification of DNA synthesis during cell proliferation.
Monoclonal Antibodies to Cell Cycle-Associated Antigens	Detection of nuclear cell cycle-associated antigens expressed only in proliferating cells.	Individual cells

Sigma-Aldrich (800) 325-3010 **www.sigma-aldrich.com**	Cell Census Plus System	Cell proliferation m asured using proprietary plasma membrane dye.	For use with flow cytometry; quantification of cell proliferation of phenotypically distinc subsets of cells withir a heterogeneous population; cell-tracking.
	In Vitro Toxicology Assay Kit, MTT based	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, and cytotoxicity.
	In Vitro Toxicology Assay Kit, XTT based	Metabolic activity of viable cells via XTT reduction to formazan salt; colorimetric assay.	Quantification of cell proliferation, viability, and cytotoxicity.
	In Vitro Toxicology Assay Kit, Acid Phosphatase based	Measurement of membrane-associated phosphatase activity; conversion of p-nitriphenyl phosphate to colored compound.	Spectrophotometric measurement of biomass (viable and nonviable cells).
	In Vitro Toxicology Assay Kit, Neutral Red based	Neutral red dye stains viable cells; colorimetric assay.	Quantification of viab cell number.
	In Vitro Toxicology Assay Kit, Sulforhodamine B based	Measurement of total protein upon sulforhodamine dye binding; colorimetric assay.	Spectrophotometric measurement of biomass (viable and nonviable cells).
	In Vitro Toxicology Assay Kit, Lactic Dehydrogenase based	Metabolic activity of viable cells measured by tetrazolium reduction to formazan derivative; colorimetric assay.	Quantification of cell proliferation, viability, and cytotoxicity.
	In Vitro Toxicology Assay Kit, Resazurin based	Metabolic activity of viable cells; bioreduction of dye converts oxidized form (blue) to fluorescent intermediate (red).	Quantification of cell proliferation, viability, and cytotoxicity.
Stratagene (800) 424-5444 **www.stratagen .com**	Quantos Cell Proliferation Assay Kit	DNA content measured using Quantos dye	Quantification of tissu culture cell numbers.

		reag nt	
Therm Labsystems (800) 522-7763 **www.labsystems.fi**	Cytotoxicity and Cell Proliferation Kit	Metabolic activity; bioluminescent detection of ATP.	Mammalian cells and cell lines in culture.
Trevigen Inc. (800) 873-8443 **www.trevigen.com**	TACS Hoechst Cell Proliferation Assay I (CPA1)	DNA content measured by A:T base pair-binding dye.	Quantification of cell number and proliferation in unfixe cells.
	TACS Hoechst Cell Proliferation Assay 2 (CPA2)	DNA content measured by A:T base pair-binding dye.	Quantification of cell number and proliferation in fixed cells.
	MTT Cell Proliferation Assay	Metabolic activity of viable cells via MTT reduction to formazan salt; colorimetric assay.	Quantification of cell number, proliferation, viability, and cytotoxicity.
Zymed Laboratories Inc. (650) 871-4494 **www.zymed.com**	BrdU Kit	DNA synthesis measured by BrdU incorporation; anti-BrdU mAb detection.	Immunohistochemistr paraffin sections.

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Last Update March 22, 2000

Apoptosis Special Interest Site

Cell Proliferation Kits

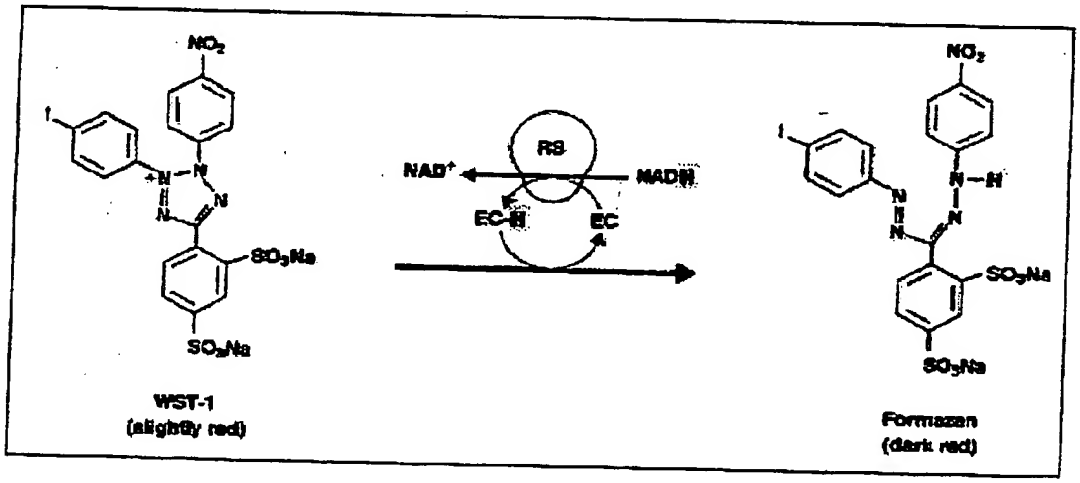
Cell Proliferation Kit I (MTT) Cat. No. 1 465 007 (2500 tests)
Cell Proliferation Kit II (XTT) Cat. No. 1 465 015 (2500 tests)
Cell Proliferation Reagent WST-1 Cat. No. 1 644 807 (25 ml = 2500 tests)

Overview

The determination of cellular proliferation, viability and activation are key areas in a wide variety of cell biological approaches. The need for sensitive, quantitative, reliable and automated methods led to the development of standard assays. Such an example is based on the capability of the cells to incorporate a radioactively labeled substance ([3 H])- thymidine), or to release a radioisotope such as [51 Cr] after cell lysis. Alternatively, the incorporation of 5-bromo-2´-deoxyuridine (BrdU) in place of thymidine is monitored as a parameter for DNA synthesis and cellular proliferation in immunohisto- and cytochemistry, in a cell ELISA and FACS analysis. (kits and reagents for these applications are available from Roche Molecular Biochemicals e. g. In Situ Cell Death Detection Kit, Cellular DNA Fragmentation ELISA).

Test Principle

Cell proliferation and viability assays are of particular importance for routine applications. Tetrazolium salts (e.g. MTT, XTT, WST-1) are especially useful for this type of analysis. These tetrazolium salts are cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the respiratory chain of the mitochondria and is active only in metabolically active cells. The figure below shows an example of this type of reaction.



The dye thus generated can be quantified e. g. by a scanning multiwell

spectrophotometer (ELISA reader) by measuring the absorbance of the dye solution at appropriate wavelengths.

Roche Molecular Biochemicals provides two test kits and a specialized reagent for analysing cell proliferation using tetrazolium salts:

- Cell Proliferation Kit MTT
- Cell Proliferation Kit XTT
- Cell Proliferation Reagen WST-1

Compared to radioactive isotope techniques, working with these products is :

- **safer** – no radioactive isotopes are used.
- **more accurate** – the absorbance revealed, strongly correlates to the cell number.
- **more sensitive** – low cell numbers are detected.
- **faster** – the use of multiwell-ELISA readers allows for processing a large number of samples.
- **easier** – no washing steps and no additional reagents are required.

Cell Proliferation Kit MTT

MTT is used for the quantitative determination of cellular proliferation and activation e.g. in response to growth factors and cytokines such as IL-2 and IL-6. It is also used for the quantification of antiproliferative or cytotoxic effects e.g. mediated by tumor necrosis factor-a or -b and for the measurement of interferon action. In cancer research the MTT assay is used for quantification of in vitro chemosensitivity of tumor cells, for the assessment of photoradiation therapy and for the screening of anticancer compounds. MTT, when dissolved in the agarose overlay is also employed to improve the visualization of virus infected cells in the plaque forming assay.

The assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells. These salt crystals are insoluble in aqueous solution, but may be solubilized by adding the solubilization solution included in the kit and incubating the plates overnight in humidified atmosphere (e.g. 37°C, 6.5% CO 2). The solubilized formazan product is spectrophotometrically quantified using an ELISA reader. An increase in number of living cells results in an increase in the total metabolic activity in the sample. This increase directly correlates to the amount of purple formazan crystals formed, as monitored by the absorbance.

Cell Proliferation Kit XTT

The XTT assay can be used for the same applications as the MTT assay. The assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolic active cells. In contrast to the purple formazan crystals which are formed in the MTT assay the XTT formazan dye is soluble in aqueous solutions and therefore can be directly quantified using a scanning multiwell spectrophotometer (ELISA reader) without the need of a solubilization step.

Cell Proliferation Reagent WST-1

The new Cell Proliferation Reagent WST-1 has several advantages compared to the above mentioned compounds. In contrast to MTT which is cleaved to water-insoluble formazan crystal and therefore has to be solubilized after cleavage, WST-1 yields water-soluble cleavage products like XTT which can be measured without an

additional solubilization step. In contrast to XTT, WST-1 is more stable. Therefore, WST-1 can be used as a ready-to-use solution and can be stored at 4°C for several weeks without significant degradation. Moreover, WST-1 has a wider linear range and shows accelerated color development compared to XTT.

Associated Documentation

We maintain a library of information including pack inserts, Material Safety Data Sheets (MSDS's), technical tips, *Biochemica* articles, and catalog information on our various products. For a list of this additional documentation, please follow these links:

- Cell Proliferation Kit (MTT)
- Cell Proliferation Kit (XTT)
- Cell Proliferation Reagent WST-1

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MTT Cell Proliferation Assay

Catalog No. 30-1010K

ATCC's MTT Cell Proliferation Assay offers a quantitative, convenient method for evaluating a cell population's response to external factors, whether it be an increase in cell growth, no effect, or a decrease in growth due to necrosis or apoptosis.

Features

- **Proven technology.** The utility of the MTT method has been documented in the literature for many different applications.
- **Accurate measurements.** The spectrophotometric procedure can detect slight changes in cell metabolism, making it much more sensitive than trypan blue staining.
- **Safer reagents.** There's no need to store or manipulate radioactive substances.
- **Easy to use.** The procedure is relatively simple and uses equipment already available in most labs.
- **Rapid processing.** Assays are run in a 96-well plate and read with a microtitre plate reader, allowing high-throughput handling of samples.
- **Convenient storage.** The kit is stable for 18 months when stored under refrigeration in the dark.

Background

The measurement of cell viability and growth is a valuable tool in a wide range of research areas. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high-throughput screening. Measuring the uptake of radioactive substances, usually tritium-labeled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances.

The reduction of tetrazolium salts is now recognized as a safe, accurate alternative to radiometric testing. The yellow tetrazolium salt (MTT) is reduced in metabolically active cells to form insoluble purple formazan crystals, which are solubilized by the addition of a detergent. The color can then be quantified by spectrophotometric means. For each cell type a linear relationship between cell number and absorbance is established, enabling accurate, straightforward quantification of changes in proliferation. Among the applications for the method are drug sensitivity, cytotoxicity, response to growth factors, and cell activation.

References

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Alley, M.C., et al. Cancer Res. 48: 589-601, 1988.
Carmichael, J., et al. Cancer Res. 47:936-42, 1987.
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Mosmann, T. J. Immunol. Methods 65: 55-63, 1983.

Frequently Asked Questions

1. **How does the MTT Cell Proliferation Assay work?**

   The MTT Cell Proliferation Assay is a colorimetric assay system which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent for approximately 2 to 4 hours, a detergent solution is added to lyse the cells and solubilize the colored crystals. The samples are read using an ELISA plate reader at a wavelength of 570 nm. The amount of color produced is directly proportional to the number of viable cells.

2. **What does the MTT system do that trypan blue cannot?**

   The MTT system is a quantitative, more sensitive test. Because there is a linear relationship between cell activity and absorbance, the growth or death rate of cells can be measured; the trypan blue test is qualitative and indicates only if a cell is alive. The MTT assay can also be adapted to high-throughput screening, whereas trypan blue tests must be read individually.

3. **How long does it take to develop purple formazan precipitate?**
   We recommend 2 to 4 hours as a starting point. This will vary somewhat among different cell types; there may be some cells that require up to 24 hours. The purple formazan has to be visible inside the cells before the detergent reagent can be added.

4. **How many cells are required to obtain an efficient reading with the MTT Cell Proliferation Assay?**

   For most tumor cells, hybridomas, and fibroblast cell lines, we recommend 5,000 cells per well to perform proliferation assays, although as few as 1,000 cells per well have been used successfully. The known exceptions are blood lymphocytes, which require approximately tenfold more cells (25,000-250,000 cells/well) to obtain a sufficient absorbance reading.

5. **Are there cell types which will not work with the MTT Cell Proliferation Assay?**

   Cells with functional mitochondria are needed to convert the tetrazolium dye into its reduced form. Most eukaryotic cells in culture, including mammalian (suspension and adhesion), plant, and yeast cell types, reduce the dye sufficiently to perform accurate assays.

6. **How do MTT Cell Proliferation Assay results compare to [3H] thymidine incorporation assays?**

   Because the MTT Cell Proliferation Assay requires less cell manipulation than [3H]thymidine incorporation assays (no cell harvesting or medium changes are necessary), the possibility of error is reduced and the standard deviation values are lower. Comparisons between [3H]thymidine incorporation and MTT assays have demonstrated less than 5% difference for determination of growth factor response.

7. **Can the MTT Cell Proliferation Assay directly replace [3H]thymidine incorporation assays?**

   Yes, the addition of dye solution can be substituted at the point in the assay when radioactive thymidine is added.

8. **Can the MTT Cell Proliferation Assay be used for cytotoxicity studies?**

   Yes, MTT Cell Proliferation Assay can be used to study cell death mediated by a cytotoxic agent.

9. How d I store the reagents?

The MTT Reagent must be kept at 4C in the dark. The Detergent Reagent can be stored at either 4C or ambient temperature. If the detergent reagent is kept at 4C, warm the bottle for 5 minutes at 37C and gently mix by inverting before use (avoid creating bubbles).

Ordering

You can order the MTT Cell Proliferation Assay from our online catalog if you have an ATCC account. Or call 800-638-6597 in the United States, Canada, and Puerto Rico or 703-365-2700 elsewhere.

This product is intended for research purposes only. It is not intended for use in humans.

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